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THE RESPONSE OF SPECIFICALLY IMMUNIZED
MICE TO REINOCULATION WITH THE VIRUS OF
ST. LOUIS ENCEPHALITIS, WITH ESPECIAL
ATTENTION TO THE DEVELOPMENT
OF MYELITIC SYMPTOMS

A PART OF A DISSERTATION SUBMITTED TO THE FACULTY OF THE DIVISION
OF THE BIOLOGICAL SCIENCES IN CANDIDACY FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY

DEPARTMENT OF BACTERIOLOGY AND PARASITOLOGY

1937

By
ENID A. COOK

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MYELITIC SYMPTOMS



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The transmission of the virus of St. Louis encephalitis to mice was first accomplished by Webster and Fite.¹ The symptom-complex, as initially described by them and subsequently observed by other workers and ourselves, consists of ruffling of the fur, tremors, ataxia and usually hyperesthesia, with death either in violent convulsions, especially where an excessive dose of virus has been given (1,000 or more cerebral lethal doses), or with profound prostration, the more typical picture.

Working with the Daily strain of the virus, generously sent to this laboratory by Dr. Muckenfuss, we have noted the most rigid adherence to the above syndrome in more than 1,000 mice inoculated with the virus in the course of various experimental procedures. Moreover, once definite nervous symptoms have appeared, the disease has almost invariably progressed to a fatal outcome.

In those animals which have had previous treatment with the virus, on the other hand, inoculation may produce an entirely different picture. Such a mouse may remain perfectly healthy after the second injection of virus, show prolonged ruffling and debility, develop typical encephalitis with death usually after a somewhat protracted incubation period, or present a sudden flaccid paralysis in one or more limbs. This last mentioned response was first observed by Armstrong and Lillie² in mice subjected to intracerebral injection after partial specific immunization by the intranasal or subcutaneous routes. The development of this sudden paralysis in our previously treated mice awakened our particular interest. After an incubation period of 5 to 14 days, the length depending chiefly on the route of inoculation but also on other factors, and without showing previous obvious symptoms, the animal may develop flaccid paralysis of one or more limbs, usually posterior. Sensitivity in the affected limb, however, is not lost. Occasionally prostration and death follow, but most often the animal remains otherwise apparently healthy, eats with good appetite and moves about its cage with the aid of whatever limbs may not be affected, or by wriggling movements of the trunk muscles when all limbs are involved. We have appended a photograph of our

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1. *Science*, **78**: 463, 1933.

2. *Pub. Health Rep.* **51**: 1069, 1936.

most severely affected mouse, no. 2, taken 34 days after the onset of the condition and showing complete paralysis of both hindlegs, partial of forelegs and tail.

We may note at this point that the injection of normal mice with suspensions of the brains or cords of paralyzed animals in our series resulted uniformly either in the development of typical encephalitis as described above with typical incubation period, symptoms and pathology, or else in the production of no symptoms whatever. Virus was found in both brain and cord, usually in somewhat higher titer in the cord. In general, the titer in both brain and cord tended to run fairly low, never more than 1 to 1,000, and to decrease rapidly, becoming nondetectable within 5 days from the onset of the paralysis.



Fig. 1.—Mouse 2.

That the disease should change thus completely in its manifestations from an encephalitis to a myelitis, seemed a striking phenomenon. St. Louis encephalitis is not normally a paralyzing infection. True paralysis may develop late in its course, usually shortly prior to death, when the animal is prostrate and the nervous system widely invaded. However, not infrequently in this state of prostration an immobile limb can be shown capable of active movement if the animal is subjected to sufficient stimulus. As our first cases were observed in a group of mice which had been inoculated intranasally in order to test for immunity after previous intraperitoneal or subcutaneous injection, we planned to study more fully the conditions under which the myelitis may be obtained.

EFFECT OF ROUTE OF INOCULATION ON THE OCCURRENCE OF PARALYSIS

In order to study the effect of the path of inoculation on the development of paralysis, groups of 20 gm. white mice purchased in the open market, were prepared by the injection of St. Louis encephalitis virus by the following routes: subcutaneous, intraperitoneal, intravenous, intranasal and intracerebral. Those inoculated subcutaneously and intraperitoneally uniformly received 0.5 cc. of a 10% suspension of virus-brain from which particles were sedimented by standing for 10 minutes. Those inoculated intravenously received 0.2 cc. of a centrifugated 5% suspension. The quantity of virus introduced in the above instances was limited by the danger of excessive trauma due to its bulk rather than that of provoking infection, for out of a total of 25 mice inoculated intravenously, 40 intraperitoneally and 96 subcutaneously,

we have observed the development of encephalitis in only one mouse, in this instance after intraperitoneal injection. The refractoriness of mice to inoculation by routes other than the intracerebral and intranasal has been noted also by Webster and Fite.³

The dosage for intracerebral and intranasal inoculation required greater caution in its determination since mice are very susceptible to infection by both paths and a quantity was desired which would not induce a fatal encephalitis but which would provoke the development of some degree of immunity. Although there was some inevitable variation in the virus titer from brain to brain, we found that a good proportion of animals would survive intranasal inoculation with 0.05 cc. of a 0.5% fresh virus-brain suspension and intracerebral inoculation with 0.03 cc. of a 1% glycerinated virus-brain suspension. As we have previously reported,⁴ although St. Louis virus may retain its infectivity for a long while in 50% buffered glycerine solution, its infective titer drops rapidly and profoundly.

At varying time intervals, small groups of the survivors of the above preparatory inoculations, usually 5 per group, were tested by the introduction of higher concentrations of virus, subcutaneously, intravenously, intraperitoneally, intranasally and intracerebrally. They were then kept under observation for a period of 2 weeks to note the occurrence of typical encephalitis or paralysis. Three sets of controls were employed. The first received a primary injection of virus-brain material after the same fashion as the test animals, but was treated with normal mouse brain suspension for its subsequent inoculation. The second set received a primary injection of normal mouse brain and a subsequent inoculation with the encephalitic material given the test groups. The third set of controls was given the first inoculation of virus and then merely kept under observation for the entire period of the test. No cases of paralysis developed in any of the controls, indicating that this condition can be produced only where a second dose of virus-brain material is introduced into animals that have had previous treatment with it.

Among the test animals certain general observations could be made. No mouse developed symptoms of any kind where the second or test dose was given by the subcutaneous, intravenous or intraperitoneal route. No mouse developed encephalitis or paralysis where both inoculations were given intracerebrally. Where the preparatory dose was given intravenously, cases of encephalitis but not of paralysis followed a test intranasal or intracerebral injection; and similarly where both inoculations were given intranasally cases of encephalitis, never of paralysis, developed. However, among a total of 40 mice given an initial subcutaneous and subsequent intranasal inoculation, three cases of paralysis developed. Of 39 mice tested intranasally after intraperitoneal inoculation, one developed paralysis. And among the group tested intracerebrally, 4 out of 15 prepared by subcutaneous injection, 1 out of 4 by intraperitoneal injection and 1 out of 10 by intranasal inoculation, developed paralysis. Pertinent data concerning all paralytic cases observed during the course of these experiments appear in table 1.

3. J. Exp. Med. **61**: 411, 1935.

4. J. Infect. Dis. **63**: 127, 1938.

From the above material, the following facts appear evident: the paralytic form of the disease may be induced fairly readily by the intracerebral inoculation of mice previously injected by subcutaneous, intraperitoneal or intranasal routes. It also may be induced, although with less frequency, by the intranasal inoculation of mice previously treated subcutaneously or intraperitoneally. Armstrong and Lillie² record their success in eliciting the condition on intracerebral reinoculation of mice previously injected subcutaneously or intranasally but failure to produce "myelitic symptoms when the second inoculation was made by the intranasal route." We feel that our demonstration of the possibility of evoking such symptoms by an intranasal

TABLE 1.—*Summary of Data on All Paralyzed Mice*

Mouse No.	Previous Treatment Routes of Inoculation and Intervening Time Periods in Days	Incubation Period (Days)	Life Span of Paralyzed Mouse (Days)	Extent of Paralysis
1	Subcutaneous (16) intranasal	14	63	Hindlegs (partial)
2	Intraperitoneal (16) intranasal	11	45	All limbs and tail
3	Intraperitoneal (14) intracerebral	11	45	Hindlegs (partial)
4	Subcutaneous (7) intranasal	10	2 ^s	Hindlegs (partial)
5	Subcutaneous (21) intranasal	12	4 ^a	Hindlegs (complete) Forelegs (partial)
6	Subcutaneous (7) intranasal	10	1 ^s	Forelegs (partial)
7	Subcutaneous (7) intranasal	11	19	Hindlegs (partial)
8	Subcutaneous (14) intranasal	11	1 ^s	Hindlegs (complete) Forelegs (partial)
9	Intraperitoneal (14) intranasal	13	2 ^s	Hindlegs (partial)
10	Subcutaneous (21) intranasal	10	3 ^s	Hindlegs (complete) Forelegs (partial)
11	Subcu. (7) Subcu. (14) intracerebral	5	1 ^s	Hindlegs and right Foreleg (partial)
12	Subcutaneous (7) intracerebral	5	1	Hindlegs (partial)
13	Subcutaneous (7) intracerebral	7	2 ^s	Hindlegs (partial)
14	Subcutaneous (7) intracerebral	7	3 ^s	Hindlegs (partial)
15	Subcutaneous (7) intracerebral	9	3	Hindlegs (complete) Forelegs (partial)
16	Subcu. (7) intravenous (14) intracereb.	5	—	Hindlegs (partial)
17	Intranas. (7) intranas. (14) intracereb.	5	10	Hindlegs (partial)
18	Intranas. (7) intracerebral	5	2 ^s	Hindlegs (complete)
19	Intraperitoneal (14) intracerebral	5	1 ^s	Forelegs (partial)
20	Subcu. (7) intranas. (18) intracereb.	10	5 ^s	Hindlegs (partial)
21	Intraperit. (7) intranas. (18) intracereb.	10	1 ^s	Hindlegs (partial)
22	Subcutaneous (7) intranasal	11	1 ^s	Hindlegs and Forelegs (partial)
23	Intranasal (7) intracerebral	7	6 ^s	Hindlegs (complete)

Legend: "a" indicates the mouse was sacrificed.
"s" indicates death was accidental.
"—" indicates survival.

reinoculation is significant both because it eliminates direct injury or introduction of foreign materials or contaminants into the central nervous system and because it offers opportunity for correlation with paths of natural infection.

Having available 34 animals which had survived two inoculations with virus, the second given 7 days after the first, we decided to determine whether a third injection would give rise to paralysis in them. For this purpose, all were inoculated intracerebrally, the route we had previously found most successful, at the end of their 2 week observation period. Five of these animals developed paralytic symptoms, cases occurring in all groups except those where one previous inoculation had been intracerebral. In all, there were 11 cases of nervous involvement among the 34 mice so treated. The cord appeared to be predominantly involved in 5 and the brain in 6.

EFFECT OF THE TIME OF REINOCULATION ON THE OCCURRENCE OF PARALYSIS

A study of the relationship of the time interval elapsing between the first and second inoculations to the development of the paralytic condition was now made. Our experiments involving reinoculation by the intranasal route were made more extensive than those by the intracerebral, since Armstrong and Lillie² have studied this latter factor. Of mice reinoculated intranasally after subcutaneous injection, paralysis developed in 1 of 10 mice after a 7 day interval, in one of 10 mice after a 16 day interval, and in one of 10 mice after a 21 day interval. One case of paralysis appeared among 9 mice inoculated intraperitoneally 16 days prior to intranasal injection. Of mice reinoculated intracerebrally, 1 of 10 treated intraperitoneally 14 days previously, 4 of 10 treated subcutaneously, and 1 of 5 intranasally 7 days previously, developed true paralysis. In view of the small number of animals tested and the several varying factors, e.g., route of inoculation and concentration of virus, no attempt is made to designate the most or least favorable time for reinoculation. It is evident, however, that the condition may follow a second injection given as early as 7 days or as late as 21 days after the first injection. We may add that, as previously noted, where mice received 2 preliminary inoculations, paralysis resulted from a third injection given as long as 25 days after the first inoculation, 18 days after the second.

INCUBATION PERIOD OF THE PARALYTIC CONDITION AND LIFE SPAN

In all instances observed, the paralysis has appeared within 5 to 14 days after the second or third injection with virus-brain suspensions. This incubation period falls within the range of the predominantly encephalitic form of the disease in mice which varies from 3 to 14 days as noted by others and ourselves. It is usually longer after intranasal than after intracerebral inoculation, which observation also holds true for the encephalitic type. Reference to table 1 shows that paralysis occurred in previously treated mice 10 to 14 days after intranasal reinoculation, 3 times on the 10th day, 4 times on the 11th, once on the 12th, once on the 13th and once on the 14th. After intracerebral injection the condition appeared in 5 to 11 days, 6 times on the 5th day, 3 times on the 7th, once on the 9th, twice on the 10th and once on the 11th.

Data concerning the life span of the paralyzed animals appears also in table 1. Unfortunately, the majority of mice had to be sacrificed shortly after the onset of symptoms when otherwise alert and in good appetite, for the sake of obtaining histological and experimental material. Of those that remained, 3 died of intercurrent infections, one on the 63rd and 2 on the 45th days, one died on the 19th and one on the 10th days without further symptoms, 2 were killed by cagemates, 2 developed pronounced prostration and died within 24 to 72 hours after the first appearance of symptoms and one was still alive and paralyzed at the termination of these experiments, after 103 days. There is probably no direct correlation between the length of life and the severity of the paralysis for our most profoundly affected animal, 2 (photograph) lived for 45 days. After 24 hours have passed there is no progression of the paralytic condition, which remains more or less static until

death, although there may be slight regression. Aside from late muscular atrophy, the picture first noted is usually maintained for the life of the animal.

EFFECT OF THE SITE OF INJECTION ON THE LOCALIZATION OF PARALYSIS

Early in the course of this work, we considered that the localization of the paralytic condition might be influenced by the site of inoculation. To test this possibility groups of 15 mice were injected subcutaneously in the right thigh, the left thigh, the left foreleg, and the peritoneal cavity. At three 7 day intervals they were tested by intranasal inoculation, the method producing least mechanical injury. Results gave no indication that the area of primary injection affects the localization of paralytic symptoms on subsequent inoculation.

An analysis of all paralytic cases observed by site of injection and subsequent symptoms (table 1) shows a similar absence of correlation. Paralysis most frequently affects the hindlegs, less frequently both hind- and forelegs, and rarely the forelegs alone or the tail, regardless of area inoculated. Furthermore, we have never observed paralysis of one side of the body to the exclusion of the other, although not infrequently a right or a left limb may be more affected than its mate. Here again, moreover, there is no correlation with the particular side inoculated.

THE POSSIBLE EXISTENCE OF LATENT VIRUS AT THE TIME OF REINOCULATION

In order to determine whether latent virus was present in the central nervous system at the time of reinoculation, the following experiment was conducted. Three groups of 15 mice each were inoculated subcutaneously, intraperitoneally, and intranasally respectively, with sublethal doses of virus. Then at weekly intervals 5 animals in each group were sacrificed, their brains and cords removed and made up separately into very thick suspensions which were in turn inoculated intracerebrally into normal mice. We failed to obtain, however, evidence of either symptoms or immunity in any of the 45 animals so treated. Of course this result does not exclude the possibility that virus may have been present, either in such small quantities as not to be detectable, or masked by neutralizing reactions in the tissues. Serum antibodies are probably not involved since we have not been able to demonstrate a significant neutralizing titer in mice up to 3 weeks after subcutaneous inoculation, and only a feeble titer after intranasal inoculation as discussed in the next section.

IMMUNOLOGICAL TESTS

Since earlier results had demonstrated that the occurrence of the paralytic form of the disease depended on previous inoculation with virus-brain suspensions and was probably the result of partial or selective immunization, we wished to study the actual degree of immunity as indicated both by the titer of specific neutralizing antibodies in the serum and the capacity to withstand reinoculation.

For this purpose, a first series of 30 white mice was injected subcutane-

ously in the region of the right groin with 0.5 cc. of a 10% suspension of fresh-virus-mouse brain. At 3 weekly intervals, 5 of the animals were bled from the heart under ether anesthesia, their serums pooled, stored until the series was completed, and then used in a neutralization test with equal quantities of 5 dilutions of virus-brain, 10^{-2} to 10^{-6} , according to the technic of Webster and Fite.⁵ The serums of normal mice and of mice proven immune to 1,000 cerebral lethal doses of virus were similarly obtained and pooled as controls. The remaining 15 of the 30 animals were inoculated intranasally, also at weekly intervals, with potent suspensions of encephalitis virus as a measure of the degree of actual immunity enjoyed. At a later date a second similar series was run with serum collected 1 and 2 weeks after intranasal inoculation and 2 weeks after intracerebral injection with sublethal doses of virus. Results for the neutralization tests appear in table 2. Also are included tests made with serum collected from three mice during the early stages of paralysis. These were titrated only against the higher virus dilutions due to the small quantity available.

TABLE 2.—*Neutralizing Power of the Serums of Mice Immunized by Various Routes*

Serums	Virus Dilutions				
	10^{-6}	10^{-5}	10^{-4}	10^{-3}	10^{-2}
Serums collected 1 week after subcutaneous inoculation	—, —	8, —	4, 7	5, 5	5, 6
Serums collected 2 weeks after subcutaneous inoculation	—, —	7, —	7, 8	5, 7	6, 6
Serums collected 3 weeks after subcutaneous inoculation	—, —	5, 9	4, 6	7, 9	5, 7
Serum collected 24 hrs. after onset of paralysis (mouse 242)	—, —	6, 7	N.T.	N.T.	N.T.
Normal mouse serum	—, —	8, —	5, 7	4, 7	4, 8
Immune mouse serum	—, —	—, —	11, —	7, 7	5, 5
Serums collected 1 week after intranasal inoculation	—, —	6, —	7, 7	6, 6	6, 7
Serums collected 2 weeks after intranasal inoculation	—, —	8, 12 ^a	7, 7	7, 9	6, 8
Serums collected 2 weeks after intracerebral inoculation	—, —	7, —	7, 7	7, 7	5, 5
Serum collected 12 hrs. after onset of paralysis (mouse 299)	7, —	7, 8	N.T.	N.T.	N.T.
Serum collected 48 hrs. after onset of paralysis (mouse 334)	—, —	6, 6	N.T.	N.T.	N.T.
Normal mouse serum	8, —	6, 8	6, 7	6, 7	5, 6
Immune mouse serum	—, —	—, —	7, 7	7, 7	7, 7

Legend: figures indicate no. of days elapsing between inoculation and death from encephalitis.

"B" indicates accidental death.

N.T. indicates no test was made.

"—" indicates survival of the animal.

We found no evidence of the presence of neutralizing antibodies in the serums of mice 1 to 3 weeks after subcutaneous injection. The serums of mice treated intranasally and intracerebrally with sublethal doses of virus possessed only a slight or questionable protective capacity. Even the control serums of our immune mice which had received several previous treatments with virus over a long period of time showed power to neutralize only 10 to 100 cerebral lethal doses of virus in contrast to that of human convalescents which may often neutralize 100 to 1,000 CLD and that of 2 rabbits which though not susceptible to the disease, we found capable of neutralizing in one case 10 to 100 and in the other 100 to 1,000 CLD. Consideration must be given the fact that the use of pooled serum permitted the dilution of serums of higher titer by those of lower titer. But even in view of this possible factor, the results obtained indicate clearly that the mouse at best is only a poor producer of serum antibodies against the virus of St. Louis encephalitis. The

serum collected from mice soon after the onset of paralysis showed no protective capacity whatever.

Let us turn now to the groups of mice prepared as above but tested for immunity by injection of strong suspensions of virus by the intracerebral or intranasal routes. Reference to table 3 demonstrates that the test animals in every group showed a lower mortality than the controls and further that the average duration of life among those affected was longer than among the controls. Since the animals developing paralysis were sacrificed early for experimental purposes, data on mortality could not be given for the groups in which they occurred.

TABLE 3.—*Response of Mice Previously Treated with the Virus of St. Louis Encephalitis to Reinoculation*

	Route of First Inoculation	Rest Period (Days)	Route of Reinoculation	Virus Concentration	No. Mice in Series	Results	Percentage Mortality from Encephalitis	Average Length of Life of Mice Fatally Affected (Days)
Test	Subcutaneous	7	Intranasal	5%	5	P* —, —, —, —	0	
Control	Not treated		Intranasal	5%	5	6, 7, 8, —, —	60	7
Test	Subcutaneous	14	Intranasal	5%	5	10, 12, 12, —, —	60	11.4
Control	Not treated		Intranasal	5%	5	8, 8, 8, 8, 12	100	8.8
Test	Subcutaneous	21	Intranasal	5%	5	10, 10, —, —, —	40	10
Control	Not treated		Intranasal	5%	5	7, 8, 9, 9, 9	100	8.4
Test	Intranasal	7	Intracerebral	1%	5	P*, P*, —, —, —	0	
Control	Not treated		Intracerebral	1%	5	5, 6, 7, 7, 7	100	6.4
Test	Intranasal	14	Intracerebral	1%	5	3 ^a , 8, 14, —, —	50	11
Control	Not treated		Intracerebral	1%	5	6, 6, 6, 7, 8	100	6.5
Test	Intracerebral	14	Intracerebral	1%	5	—, —, —, —, —	0	
Control	Not treated		Intracerebral	1%	5	6, 6, 6, 7, 8	100	6.5

Legend: figures in results indicate no. of days elapsing between inoculation and death from typical encephalitis.

"P*" indicates development of paralysis and death of the mouse.

"a" indicates accidental death.

"—" indicates survival of the animal.

PATHOLOGY

The brains and cords of mice that died of the infection and of those that were sacrificed showed no gross pathological changes beyond varying degrees of vascular congestion. All histological material was fixed in piciformol and stained with eosinemethylene blue or hematoxylin picro-fuchsin. The microscopic pathology in the brains and cords of those mice that died with typical encephalitis followed closely the general pattern already fully described by McCordock and coworkers,⁶ Weil,⁷ Webster and Fite,⁸ Smadel and Moore⁹ and others, and need not be repeated here. In mice with the paralytic form of the disease, the same pathological features also appeared, differences occurring only in the extent and distribution of lesions. While there was inflammatory change and scattered nerve-cell degeneration throughout the central nervous system in these latter cases, the most severe manifestations were regularly located in the cord. Our sections showed very pronounced vascular congestion, perivascular and diffuse infiltration with round cells, proliferation of microglia, and nerve cell degeneration mainly in

6. J.A.M.A. **103**: 822, 1934.

7. Arch. Neurol. & Psychiat. **31**: 1139, 1934.

8. J. Exper. Med. **61**: 103, 1935.

9. Am. J. Path. **10**: 827, 1934.

the anterior horns. No essential differences in the pathological changes in the cord were observed in mice showing paralysis after intranasal and after intracerebral injection.

DISCUSSION

The facts just recorded have demonstrated that symptoms and pathological changes pointing to a predominant involvement of the cord may be produced in mice by reinoculation with suspensions of the brains of mice dead of St. Louis encephalitis, and have outlined, further, the conditions under which such symptoms may be obtained. However, the possibility of the existence of contributing or even causative agents other than the St. Louis encephalitis virus should not be ignored. Other factors that might reasonably be suspected of playing a part are: (1) trauma, (2) bacterial infection, (3) the accidental presence of another neurotropic virus in the inoculum, (4) the activation of a latent virus already present in the stock experimental animals, and (5) spontaneous variation in the St. Louis virus itself. We will now consider these possibilities in order.

Trauma.—Armstrong and Lillie² are inclined to give some weight to the possible influence of trauma on the development of the paralytic type of the disease since they obtained cases only after intracerebral inoculation. In as much as we, however, have readily obtained cases when there was no direct mechanical injury to the central nervous system, i.e., after reinoculation by the intranasal route of animals previously treated subcutaneously or intraperitoneally, we feel that this factor can safely be ruled out.

Bacterial infection.—Bacterial infection also has been shown not to be involved. Examination of all inocula prior to injection, and of all necropsy material by direct smear and by aerobic and anaerobic cultivation has not revealed any constant or significant bacterial agent. Furthermore, histological study of sections of brain and cord has not resulted in the detection of bacteria. In addition, 2 rabbits and 3 guinea pigs injected intracerebrally with concentrated suspensions of the brain and cords of paralyzed mice developed no symptoms whatever. And finally, in the course of another investigation,¹⁰ we were unable to obtain evidence of any alteration in the pathogenesis of St. Louis encephalitis in mice as a result of the inoculation of these animals with a wide variety of bacterial pathogens and saprophytes.

The accidental presence of another neurotropic virus in the inoculum.—A further possible explanation is that some other neurotropic virus might have been picked up accidentally in the course of experimentation and carried along routinely in the stock virus-brains. The following facts, however, are not consistent with such an hypothesis. Myelitic symptoms appear only upon reinoculation of mice, never after a first injection even where the animals have been kept under observation for as long as 2 months. Also, virus stored in glycerine prior to the initiation of this study still gave perfect cross-immunity with that obtained from paralyzed mice. And finally, serums from human St. Louis encephalitis convalescents neutralized virus from both encephalitic and paralytic cases.

10. Cook, E. A.: to be published.

The activation of latent virus.—The possibility that merits our most serious consideration is that the condition might be the result of the activation of a latent spontaneous mouse virus due to weakening of the normal protective mechanism under the attack of the virus of St. Louis encephalitis. Of the known spontaneous virus diseases of mice, the only one that presents sufficiently similar symptoms and pathological changes to allow any confusion with the myelitic form of St. Louis encephalitis is "spontaneous encephalomyelitis" described by Theiler.¹¹ Unfortunately we failed to obtain any of this virus for cross-immunity and other comparative studies that we should have liked to undertake. We feel, however, that the 2 conditions may be regarded as distinct in view of the general evidence already presented relating to our failure ever to observe spontaneous paralysis in any of our stock mice, or in any mouse surviving a single injection with St. Louis encephalitis virus, to the reproduction of typical encephalitis on inoculation of normal mice with the brains or cords of those showing paralysis, and to the essential similarity of the pathological features of both the encephalitic and the myelitic forms of the St. Louis disease. Furthermore, there are certain specific differences in incubation period, age susceptibility and persistence of virus in the central nervous system of infected animals, between spontaneous encephalomyelitis as described by Theiler and the predominantly myelitic form of St. Louis encephalitis as described by Armstrong and Lillie, and ourselves.

Variation of the St. Louis virus itself.—We come now to a consideration of our last mentioned possible explanation for the occurrence of the condition, namely, variation in the St. Louis virus itself. It is conceivable that St. Louis encephalitis virus might have undergone a variation manifested by new cellular affinities as a result of growth in the tissues of partially immune animals, even as certain bacteria and other viruses have been shown to do. But in as much as normal mice injected with the brains or cords of paralyzed animals develop typical encephalitis, we should have to postulate either a rapid reversion or else the transformation of only a small amount of the total virus content, which remains masked on injection into normals. Perhaps serial transfer of virus to partially immunized animals might result in the selection and preservation of such a variant strain. However, it is more in keeping with our present conceptions to conclude that the change in symptoms is due rather to an alteration in the response of the animal as a result of previous immunization than to a change in the virus itself.

SUMMARY

Intracerebral or intranasal reinoculation of mice 7 to 21 days after 1 or 2 previous injections with the virus of St. Louis encephalitis by either the subcutaneous or intraperitoneal route may give rise to a sudden flaccid paralysis of the limbs with fatal or nonfatal outcome. Similar results may be obtained by the intracerebral reinoculation of mice after 1 or 2 previous intranasal inoculations.

The incubation period of the paralytic condition is 5 to 14 days, being shorter (5 to 11 days) after intracerebral injection than after intranasal (11 to 14 days).

11. J. Exper. Med. 65: 705, 1937.

Suspensions of the brains or cords of such paralyzed animals give rise to typical encephalitis on injection into normal mice. Virus exists usually in slightly higher relative titer in the cord than in the brain, but is in comparatively low titer in both and disappears rapidly.

The localization of the paralytic symptoms is not correlated with the site of injection, and virus is not detectable in the central nervous system 7, 14 or 21 days after the injection of a nonlethal dose.

The serum of mice given a single subcutaneous or intranasal nonlethal injection with virus possesses little or no neutralizing capacity. However, a certain degree of immunity is shown to exist in animals so treated by their resistance to subsequent inoculations with virus rapidly lethal to controls.

The chief pathological findings in the cords of paralyzed mice are vascular congestion, perivascular and diffuse round cell infiltration, proliferation of microglia and nerve cell degeneration, especially in the anterior horns.

